SOME PHYSIOLOGICAL EFFECTS OF NH_4^+ AND Al^{3+} ON PINE FOREST ECOSYSTEMS

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INTRODUCTION

Due to intensive agriculture activities in The Netherlands a considerable deposition of ammonium sulphate occurs on vegetation and soil (Aalst van, 1984). This high deposition with NH_4^+ results in an accumulation of NH_4^+ in the soil and in a simultaneous leaching of the essential nutrients as K^+, Ca^{2+}, and Mg^{2+} from the soil (Roelofs et al., 1985). The overall effect is an increase in the ratios of NH_4^+ to K^+, Ca^{2+} and Mg^{2+} and an increase in the ratio of Al^{3+} to Ca^{2+}. Roelofs et al. (1985) clearly demonstrated a good correlation between these increased ratios and the condition of the pine forests.

Increased ratios of NH_4^+ to K^+ and Mg^{2+} may have strong effects on the nutrient uptake, since it is known that these increased ratios lead to inhibition of the uptake of K^+ (Smith and Epstein, 1964) and Mg^{2+} (Jacob, 1958). Increased Al^{3+} to Ca^{2+} ratios may lead to damage of the root system. Ulrich (1983) mentions an Al/Ca ratio ≥ 5 in the soil solution as very critical for root damage.

In this study the role of increased NH_4/K ratios on the growth of some ectomycorrhizal fungi is examined, since it has been shown that particularly in areas with a high NH_4^+ deposition, the total number of symbiotic fungi has been strongly decreased (Arnolds, 1985). Symbiotic fungi are of great importance to the tree, since they expand the absorption area of the root considerably and all the nutrients must pass the fungal mantle before they reach the rootsystem (Harley and Smith, 1983). It has further been examined whether there were some direct effects of NH_4^+ and Al^{3+} on the nutrient uptake in isolated ectomycorrhizal fungi, in seedlings of *Pinus sylvestris* and in four year old *P.nigra*.

RESULTS AND DISCUSSION

The effect of NH_4^+ on the extension growth of various ectomycorrhizas.

The effect of NH_4^+ on the extension growth of mycorrhizal fungi was studied in pure culture on a solid MMN medium (Marx, 1969), except that the salts were added at an one-fourth strength.

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Table I: The effect of increasing NH_4/K ratios on the extension growth of various ectomycorrhizal fungi at pH 5.5. Growth is expressed as % of the growth in the logarithmic phase at an NH_4/K ratio of 1.

	NH_4/K ratio				
	1	5	10	20	40
Amanita muscaria	100%	45	19	24	26
Amanita rubescens	100%	32	19	22	19
Cenococcum geophilum	100%	25	23	20	25
Hebeloma crustuliniforme	100%	7	6	8	9
Laccaria laccata	100%	38	33	28	22
Laccaria proxima	100%	57	42	30	29
Pisolithus tinctorius	100%	73	60	53	49
Rhizopogon luteolus	100%	85	53	45	41
Suillus bovinus	100%	97	78	76	65
Suillus granulatus	100%	52	36	36	34
Suillus luteus	100%	44	29	29	34
Suillus variegatus	100%	111	99	51	27
Thelephora terrestris	100%	73	69	--	24

As shown in Table I the growth of all of the ectomycorrhizal fungi examined becomes inhibited by increasing the NH_4/K ratio. For *Cenococcum geophilum* and *Hebeloma crustuliniforme* the NH_4/K ratio is already very critical at low levels. Increasing this ratio from 1 to 5 caused a severe inhibition of the extension growth, whereas a further increase of this ratio resulted only in a slight additional increase or no further increase in inhibition of the extension growth. In general it can be concluded that the inhibition of the extension growth is 60-80% at NH_4/K ratios of 10 or more.

The effect of NH_4^+ on the K^+ ($^{86}Rb^+$) and $H_2{}^{32}PO_4^-$ uptake in an isolated ectomycorrhizal fungus.

The effects of NH_4^+ on the nutrient uptake were studied with liquid cultures of *Rhizopogon luteolus* using radio-active isotope techniques

(Theuvenet and Borst-Pauwels, 1976), with $^{86}Rb^+$ as a tracer for K^+.

The effect of increasing NH_4/Rb ratios on the uptake of 100 µM Rb^+ in *R. luteolus* is shown in Table II

Table II: The effect of increasing NH_4/Rb ratios on the uptake of 100 µM Rb^+ in *R. luteolus* at pH 4.0.

NH_4^+ (µM)	NH_4/Rb	Rb^+ uptake (%)
0	0	100
100	1	59
200	2	27
1000	10	11
5000	50	3

With increasing NH_4^+ concentrations the Rb^+ uptake becomes increasingly inhibited. It was also examined whether NH_4^+ inhibited the Rb^+ uptake at other Rb^+ concentrations. The results are shown in Table III.

Table III: The effect of NH_4^+ on the Rb^+ uptake in *R. luteolus* at pH 4.0.

Rb^+ (mM)	+ 100 µM NH_4^+ % of control	+ 1000 µM NH_4^+ % of control
0.001	49	6
0.01	54	6
0.025	50	6
0.1	61	9
0.25	71	9
1.0	84	24
10.0	86	77

At low Rb^+ concentrations the greatest percentage of inhibition in Rb^+ uptake was found on adding NH_4^+. The inhibition was partly reversed when the Rb^+ concentration was raised. This indicates that NH_4^+ competitively inhibits the Rb^+ uptake.

The effect of NH_4^+ on the phosphate uptake is shown in Table IV.

Table IV: The effect of NH_4^+ on the phosphate uptake in *R. luteolus* at pH 4.0

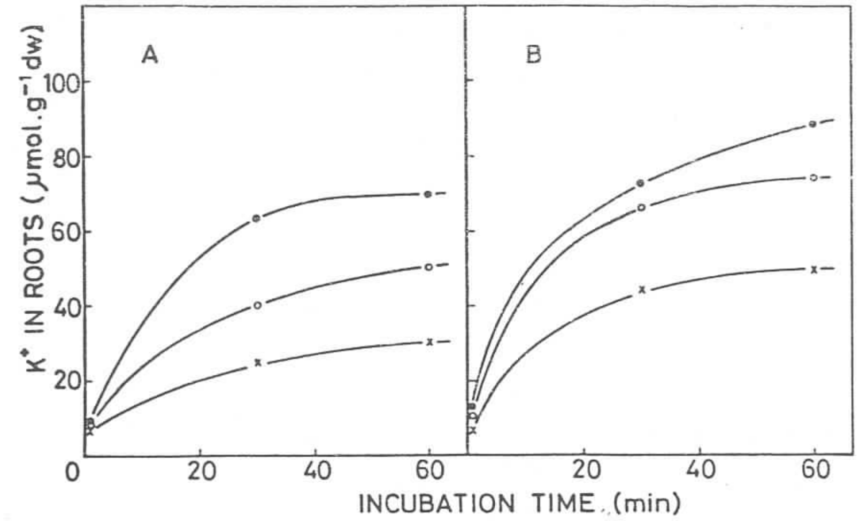
$H_2PO_4^-$ (µM)	+ 1000 µM NH_4^+ % of control
1	45
10	38
40	75
100	60

This table shows that the phosphate uptake is also inhibited by NH_4^+, although this type of inhibition is non-competitive. This means that the inhibition of the phosphate uptake is independant of the phosphate concentration applied.

The effect of NH_4^+ on the uptake of K^+ ($^{86}Rb^+$) in P. sylvestris seedlings. Effects of mycorrhizal infection.

In this study seedlings of approximately 7 months old were used of which a part was inoculated with the fungus *R. luteolus*.
Fig. 1 shows that the uptake of K^+ by mycorrhizal roots is higher than that by non-mycorrhizal roots. Increasing of the NH_4^+ concentration resulted into inhibition of K^+ uptake. The percentage of inhibition by NH_4^+ is about the same in mycorrhizal and non-mycorrhizal roots, although the accumulation level still remains higher in the mycorrhizal roots. Since mycorrhizal growth is inhibited by NH_4^+ the effect of NH_4^+ may be two-fold. First an inhibition of the K^+ uptake may be observed and second, due to the disappearance of the fungi, a still greater inhibition in K^+ uptake may be found.

Fig. 1: The effect of NH_4^+ on the K^+ uptake in *P. sylvestris* seedlings at pH 4.0. A: non-mycorrhizal and B: mycorrhizal seedlings. ●: control $NH_4/K = 1$, o: $NH_4/K = 10$, x: $NH_4/K = 50$.



Effects of NH_4^+ and Al^{3+} on the uptake of K^+, Ca^{2+} and Mg^{2+} in four year old P. nigra.

P. nigra was grown in hydroculture using a continuous flow of growth medium. The effects of NH_4^+ on the nutrient uptake are shown in Table V. The uptake of nutrients was studied from the retention of nutrients from the growth medium.

Table V shows, as found earlier, that the K^+ uptake becomes inhibited as the NH_4/K ratio increases. The same phenomenon was, however, also found for the Ca^{2+} and Mg^{2+} uptake. At an NH_4/K ratio of 50 the plants do not accumulate any Ca^{2+} or Mg^{2+} but even start to loose their Ca^{2+} and Mg^{2+}.

The effects of Al^{3+} on the nutrient uptake are shown in Table VI.

Table V: The effect of NH_4^+ on the cumulative uptake of K^+, Ca^{2+} and Mg^{2+} in *P. nigra* at pH 4.0. (+) and (-) refer to uptake or release respectively The experimental period was 16 weeks.

NH_4/K ratio	K^+ (µM)	Ca^{2+} (µM)	Mg^{2+}
1	+ 680	+ 220	+ 190
5	+ 620	+ 125	+ 95
10	+ 695	+ 40	+ 85
50	+ 110	- 110	- 40

Table VI shows that there are no pronounced effects of Al^{3+} on the K^+ uptake, but that Al^{3+} influences the Ca^{2+} and Mg^{2+} uptake. Increase of the Al/Ca ratio results in a progressive inhibition of the Ca^{2+} and Mg^{2+} uptake, the plants start even to loose their Ca^{2+} and Mg^{2+}.

Table VI: The effect of Al^{3+} on the cumulative uptake of K^+, Ca^{2+} and Mg^{2+} in *P. nigra* at pH 4.0. See further legend to Table V.

Al/Ca ratio	K^+ (µM)	Ca^{2+} (µM)	Mg^{2+} (µM)
0	+ 680	+ 220	+ 190
1	+ 1100	+ 15	- 85
5	-	- 80	- 95
10	+ 755	- 80	- 75

CONCLUSIONS

1. The growth of the ectomycorrhizal fungi is severely inhibited by increased NH_4/K ratios.
2. NH_4^+ excerts a direct effect on the nutrient uptake in isolated corrhizal fungi and pine trees. The K^+, Ca^{2+}, Mg^{2+} and phosphate uptake become in-

hibited by NH_4^+.

3. Al^{3+} also excerts a direct effect on the nutrient uptake in *P. nigra*. The K^+ uptake seems not to be affected, while there is a strong inhibition of the Ca^{2+} and Mg^{2+} uptake, even an efflux of these ions was found.
4. Due to $(NH_4)_2SO_4$ deposition the pine forests may become suffering from shortage of nutrients. Ultimately the pines may die due to deficiency of essential nutrients and to an increased susceptability to secondary stress factors.

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